Title: The potential of fecal calprotectin as an objective marker to discriminate hospitalized patients with acute severe colitis from outpatients with less severe disease

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Abstract

Background: Acute severe colitis(ASC) is conventionally diagnosed by Truelove and Witts' criteria which are non-specific and can be affected by other pathologic conditions. Fecal calprotectin(FCP) is a gut-specific marker of inflammation which can predict short-term outcomes in patients with ASC. We aimed to define the role of FCP in the diagnosis of ASC.

Methods: This prospective observational cohort study included adult patients (>18 years) with ulcerative colitis(UC) for whom FCP was measured and were under follow-up from April 2015 –December 2016. Patients were divided into two cohorts:1)all consecutive hospitalized patients with ASC as defined by Truelove and Witts' criteria; 2)outpatients with active UC (defined by Mayo score) who did not fulfill Truelove and Witts' criteria. FCP levels were compared between the two cohorts and a cutoff for FCP to diagnose ASC was determined.

Results: Of 97 patients, 49 were diagnosed with ASC (mean age: 36.1$\pm$11.9 years, 36 males) and 48 with active UC (mean age: 37.9$\pm$12.4 years, 25 males). Median FCP levels were significantly higher in patients with ASC [1776(952 – 3123) vs 282(43 – 568) µg/g, p<0.001] than mild to moderately active UC(n=48) or moderately active UC [n=35, 1776(952 – 3123) vs 332(106 – 700) µg/g, p<0.001]. A FCP cutoff of 782 μg/g of stool had excellent diagnostic accuracy, with an area under the curve of 0.92(95%CI:0.87–0.97), sensitivity of 84% and specificity of 88% to differentiate ASC from active UC.

Conclusion: FCP could differentiate ASC from mild to moderate patients with UC, but requires validation before clinical use.

Key words: Fecal calprotectin; acute severe colitis; ulcerative colitis; Mayo score; hospitalization

Introduction

Acute severe colitis (ASC) is a medical emergency that requires hospitalization and time bound management. It complicates the course of ulcerative colitis in up to 25% patients with one-third of these episodes being the first episode of UC[1,2]. ASC is diagnosed based upon Truelove and Witts' criteria which includes 6 or more bloody bowel movements/ day along with one of raised ESR (> 30 mm/hr), fever (>37.8), tachycardia (>90/min) or anemia (haemoglobin <105 g/l)[3]. This definition of ASC has stood the test of time and has been uniformly used across studies of ASC through-out the globe. But some of the criteria used to define ASC are subjective and are influenced by many other variables other than ASC. ESR can be elevated in patients with anemia, pulse rate can increase for many reasons, fever can occur because of concomitant infection which is common in developing countries and the average mean haemoglobin in developing countries is lower compared to developed countries[4,5]. Therefore, all the additional criteria used to define ASC have some short comings which may lead to over-diagnosis of ASC and increase hospitalization and the cost of treatment. There is a need for an objective parameter to improve the diagnosis of ASC.

Fecal calprotectin (FCP), a neutrophilic cytosolic protein[6], has been used as a marker of disease activity in patients with inflammatory bowel disease (IBD)[7] and has been found to discriminate between mild, moderate and severe endoscopic appearance of UC[8], predict relapse in patients of UC in clinical remission[9,10,11], and a fall in serial FCP levels has been shown to predict response to infliximab therapy in UC[12]. We recently documented that day 3 FCP in patients with ASC could predict short-term outcomes with reasonable accuracy[13]. However, no study has evaluated the value of FCP in the diagnosis of ASC. We hypothesize that FCP levels would be significantly higher in ASC compared to mild to moderate or severely active UC, and could be used as an objective parameter to diagnose ASC.

Material and methods

Patients

This prospective observational cross-sectional study included adult patients (>18 years) of ulcerative colitis, for whom fecal calprotectin was done, and were following up at All India Institute of Medical Sciences, New Delhi, India from April 2015 to December 2016. Patients aged >75 years, pregnant women, patients with a history of diabetes mellitus, chronic kidney disease, hypertension, or coronary artery disease and patients refusing consent were excluded from the study. The study protocol was approved by the institutional ethics committee (IESC/T-215/05.05.15, dated May 5th 2015) of AIIMS. Written, informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Study design

The patients were divided into two cohorts for further analysis. The first cohort included all consecutive patients with ASC defined by Truelove and Witts' criteria who were hospitalized at AIIMS. The second cohort included patients with mild to moderate disease activity who did not fulfill Truelove and Witts' criteria for ASC, and were managed as outpatients. Clinical information was collected, incorporating all baseline characteristics as well as treatment details. Data were collected for patient demographics, disease duration, type of disease and severity, treatment given and outcome. Fecal samples were collected for measuring FCP levels, both in patients with ASC and in outpatients with active UC at presentation. Endoscopy and collection of fecal sample were done on the same day (fecal sample was collected before endoscopy).

Definitions

Ulcerative colitis: Diagnosis based on clinical, radiologic and histological criteria (11). Patients with index presentation of ASC who later turned out to be infection or Crohn's colitis were excluded[14].

Acute severe colitis: Based on Truelove and Witts' criteria (6 or more stools with blood and one or more of following: haemoglobin<105 g/L, ESR >30 mm/hr, fever >37.8ºC, or tachycardia >90/minute[3].

Disease extent: Maximum macroscopic extent at colonoscopy, using the Montreal classification[15]. For patients presenting with ASC at diagnosis, extent was determined from the first colonoscopy after discharge or the surgical specimen if they underwent colectomy.

Disease activity: Disease activity in patients with UC (mild/moderate/severely active) was assessed using the Mayo Score: 3 – 6 for mild, 7 – 9 for moderate and 10 – 12 for severe disease activity[16].

Measurement of Fecal calprotectin levels: Stool samples were collected and frozen at -20° C. Fecal calprotectin levels were measured by ELISA based methods using KAPEPKT849 kit (DIA source Immuno Assays S.A. – Belgium). The person performing measurement of FCP levels (VS) was blinded to the clinical and endoscopic details of the patients.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median (inter-quartile range) as appropriate and categorical variables are expressed as frequency (percentage). Categorical variables were compared using chi square test. The continuous variables among the two cohorts were compared using unpaired t test or Mann Whitney U test depending upon normal or non-normal distribution. Median FCP values were compared between the two cohorts and a receiver operating characteristics (ROC) curve was plotted to determine the optimal cut-off of ASC to differentiate ASC from outpatients with active UC who did not meet Truelove & Witts' criteria. A P value of < 0.05 was considered as statistically significant. Data analysis was done using SPSS version 24.

Results

A total of 50 consecutive patients with 54 episodes of ASC were hospitalized in AIIMS from June 2015 to November 2016. Five patients were excluded and 49 episodes of ASC were included (Figure 1). In the other cohort, 50 patients with ulcerative colitis had FCP testing performed, of these 2 patients were in remission and were excluded. So, a total of 97 patients with ulcerative colitis were included in this study (Figure 1).

Baseline clinical and demographic characteristics

There was no difference in the mean age of patients with ASC compared to outpatients with active UC (37.9 + 12.4 years vs 36.1+ 11.9 years, p=0.47). There were more males among patients with ASC than patients with active UC not meeting ASC diagnostic criteria (74% vs 50%, p=0.01). The median disease duration was longer in patients with ASC [36 (15 – 60) vs 12 (8 – 31), p=0.001] months. No patient with ASC had proctitis and the frequency of pancolitis was higher in ASC than those without ASC (Table 1). The median Mayo score among patients with ASC was significantly higher than the out-patient UC cohort [11 (11 – 12) vs 8 (6 – 8.75), p<0.001]. Among the non-ASC patients, a subgroup of patients with moderately active UC (n=35), and another subgroup among moderately active UC (with Mayo stool frequency sub-score=3, and rectal bleeding sub-score=2, n=23) had almost similar features as that of the entire outpatient UC cohort (Table 1).

Comparison of FCP between acute severe colitis and mild to moderately active ulcerative colitis

The median FCP level among patients with ASC was significantly higher than for outpatients with mild to moderately active UC [1776 (952 – 3123) vs 282 (43 – 568)µg/g, p<0.001], or the subgroup with moderately active UC(n=35) [1776 (952 – 3123) vs 332 (106 – 700) µg/g, p<0.001], and those with moderately active UC and Mayo stool frequency sub-score of 3 and rectal bleeding score of 2 (n=23) [1776 (952 – 3123) vs 420 (123 – 700) µg/g, p<0.001]. The difference between ASC and active UC remained similar when patients of proctitis were excluded from the outpatient UC cohort [1776 (952 – 3123) vs 278 (44 – 569) µg/g, p<0.001].

The ROC curve for FCP to differentiate ASC from outpatients with mild to moderately active UC had an area under curve (AUC) of 0.92 (95% CI: 0.87 – 0.97) (Figure 2). A FCP cut-off of 782 μg/g of stool had a sensitivity of 84%, specificity of 88%, positive predictive value (PPV) of 87% and negative predictive value (NPV) of 84% for a diagnosis of ASC (Table 2).

Sub-group analysis to explore the value of FCP for differentiating ASC from patients with moderately active disease (Mayo score >6) had a similar diagnostic accuracy, with an AUC of 0.9 (95% CI: 0.84 – 0.97) (Figure 3a). Again, FCP cut-off of 782 μg/gram of stool had a sensitivity of 84%, specificity of 86%, PPV of 89% and NPV of 79% to diagnose ASC (Table 2).

We did another subgroup analysis by including only those patients, among patients with moderate UC, who had a Mayo stool frequency sub-score of 3 and had rectal bleeding sub-score of 2 (n=23). In this subgroup analysis also FCP had almost similar diagnostic accuracy with an AUC of 0.89 (95% CI: 0.82 – 0.97) (Figure 3b). FCP cut-off of 747 μg/gram of stool had a sensitivity of 84%, specificity of 83%, PPV of 91% and NPV of 70% to diagnose ASC (Table 2).

Comparison of fecal calprotectin in patients with acute severe colitis according to the number of additional Truelove and Witts' criteria

There was a progressive, although statistically non-significant rise in the median FCP levels with an increase in number of additional Truelove & Witts' criteria (Table 3). The median FCP levels in patients with only 1 additional criterion was 994 (IQR: 596 – 2435) μg/g, whilst it was 1783 (IQR: 1311 – 3940) μg/g of stool in patients with > 1 criteria (P= 0.07) (Figure 4)

Discussion

Acute severe colitis manifests itself through systemic features of inflammation when the colonic inflammation is particularly severe: fever, tachycardia, elevated ESR/CRP and anemia in addition to stools with blood[3]. However, these are non-specific features which can be altered by many other physiologic and pathologic processes, which can affect the diagnosis of ASC. A feature which is specific to colonic inflammation and also characterizes the systemic component would be ideal to allow an objective diagnosis of ASC. Fecal calprotectin is a marker which may satisfy these criteria[613].

FCP was significantly higher in patients with ASC compared to outpatients with mild to moderately active UC as defined by the Mayo Clinic score (8(IQR:6 – 8.25)). This difference persisted even when mild cases and cases with proctitis were excluded from outpatient UC cohort to make the control population more homogenous. Even among the patients with ASC, there was a rising trend of FCP (although statistically insignificant because of small numbers) with an increasing number of additional Truelove Witt's criteria, the markers of a systemic inflammatory response. An increasing number of additional Truelove & Witts' criteria represents increasing severity of inflammation and is associated with an increased need for colectomy[1].

This indicates that FCP in addition to being a gut-specific marker might also quantify the severity of systemic inflammation in ASC and it is this feature of FCP which could help in the objective diagnosis of ASC. The diagnostic threshold of FCP >782 μg/g for differentiating ASC from levels of active UC showed excellent accuracy, with an AUC of 0.92, a sensitivity of 84% and specificity of 88%. Even when we excluded mild cases from analysis, the diagnostic accuracy remained similar, with an AUC of 0.9, sensitivity of 84% and specificity of 86%, indicating that this threshold discriminates between those with a systemic inflammatory response needing hospital admission and those without, who can be managed as outpatients. Therefore, in an appropriate clinical context (a patient with UC and $\geq$6 bloody stools/day), an elevated FCP (>782 μg/gram) indicates a diagnosis ASC with reasonable accuracy. It may qualify for a

point of care test in the clinic to diagnose ASC, although the delay in receiving the results may limit its value in determining the need for admission.

The study, although prospective, inevitably has limitations. The present results are from a relatively small number of patients from a single center tertiary care institute. We did not have patients with severe UC (Mayo score $\geq$ 10) in the outpatient cohort, and this would affect the validity of our results, as it is in this cohort where one needs to decide on hospitalization. But Mayo score is a combination of 4 parameters which includes endoscopic activity and physician global assessment in addition to stool frequency and rectal bleeding, and 66% patients with moderate disease activity had rectal bleeding sub-score of 2 and stool frequency sub-score of 3 (> 4 stools above normal stool frequency). Patients with ASC have >6 bloody stools/day with systemic feature, and short of systemic features, the latter subgroup would most closely resemble ASC in terms of stool frequency and rectal bleeding. Even in this cohort (n=23), the FCP cut-off of 747 μg/gram had an excellent diagnostic accuracy to diagnose ASC. Reporting outcomes for both the cohorts (ASC and outpatient UC) would have added strength to the paper. However, this study was done with an aim to establish the role of FCP in differentiating ASC from outpatient UC. Moreover, the sample size is small for any meaningful results on outcomes. Ideally FCP should be tested in a specific cohort: those patients with active UC and a bloody stool frequency $\geq$6/day and related to outcomes.

To conclude FCP >782 μg/g in a patient with active UC can discriminate patients with severe disease requiring hospitalization from outpatients with less severe disease with a high diagnostic accuracy. Fecal calprotectin therefore has the potential to develop as an objective marker to diagnose acute severe colitis.

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Author contributions

Guarantor of article: Vineet Ahuja

SK: Data acquisition, data analysis and drafting the manuscript

SJ and SG: Data acquisition and critical revision of the manuscript for important intellectual content

VS: Analysis of Fecal calprotectin

SB, DPY, PS, SP, NRD, SPLT, GM: critical revision of the manuscript for important intellectual content

VA: Study concept and design, drafting the manuscript and critical revision of the manuscript for important intellectual content

All authors have made significant contributions to the manuscript and agree with the content of the manuscript.

Figure legends

Figure 1: Study flowchart

Figure 2: ROC curve to determine fecal calprotectin cut-off to differentiate acute severe colitis (n=49) cases from entire outpatient ulcerative colitis cohort (n=48)

Figure 3: ROC curve to determine fecal calprotectin cut-off to differentiate acute severe colitis (n=49) cases from A) outpatient ulcerative colitis with moderate disease activity(n=35); B) outpatient ulcerative colitis with moderate disease activity and Mayo stool frequency sub-score of 3 and rectal bleeding sub-score of 2 (n=23)

Figure 4: Box plots comparing median fecal calprotectin levels in acute severe colitis patients according to number of additional Truelove and Witt's criteria (1 vs >1)

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